<Division of Biochemistry> Molecular Biology

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- Molecular Biology -

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Prof BANKAITIS, V.A. — University of North Carolina, Chapel Hill, USA, 26 April 2004
Prof QU, L.-J. — Peking University, Beijing, China, 6 - 7 August 2004

Scope of Research

This laboratory aims at clarifying the framework of regulatory network between genetic programs and environmental stress responses through the study on structure-function relationships of genetic materials and cellular proteins in higher plants and pathogens. The major subjects are mechanisms involved in intracellular signal transduction and regulation of gene expression responsive to environmental stimuli, differentiation and development of plant organs, and plant-microbe interaction. As of December 2004, study is being concentrated on the two-component response regulators involved in cytokinin signaling, HD-Zip proteins required for phospholipid signaling, COP9 signalosome modulating protein degradation, and cyclines and CDKs controlling cell cycle.

Research Activities (Year 2004)

Presentations

Sequence structure recognized by the *Arabidopsis* response regulator ARR1, Taniguchi M, Aoyama T, Oka A; Expression analysis of the *AtPLDζ2* gene in *Arabidopsis thaliana*, Yamamoto Y, Ohashi Y, Oka A, Aoyama T, 2004 Ann Meeting of Jpn Soc Plant Physiol, 27 - 29 March (Hachioji).

Phospholipase D controlling plant cell morphogenesis, Aoyama T, 2004 Spring Symposium of Mol Biol Soc Jpn, 19 - 20 May (Nara).

Targets of ARR1, Aoyama T, International Workshop on Cytokinin Metabolisms, Signaling and Function, 10 July (Berlin, Germany).

Interaction between CSN1 (COP9 Signalosome subunit 1) and components of transcription/RNA processing complexes SAP130, DdX15/hPrp43/mDEAH9, and CFIm68, Menon S, Tsuge T *et al*., 2004 FASEB Summer Research Conference at Vermont Academy, 14 - 19 August (Saxtons River, VT USA).

Contribution of phospholipase D to morphogenesis of plant epidermal cells, Oka A, Ohashi Y, Aoyama T, 2004 Ann Meeting & Symposia of Genetics Soc Jpn, 27 - 29 September (Suita).

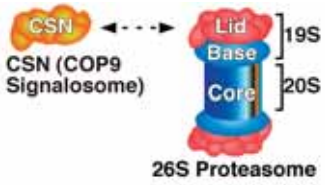
Role of phospholipase D in root-hair cell morphogenesis, Ohashi Y *et al*., European Plant Science Organization Conference: Interactions in Plant Biology: cells, plants and communities, 10 - 14 October (Ischi, Italy).

Hunting target genes of *Arabidopsis* transcription factors, Aoyama T, Workshop at University of Rome La Sapienza, 18 October (Rome, Italy).

The Challenge on Revealing the Novel Regulation of COP9 Signalosome; a Master Regulator of Plant Signal Transduction

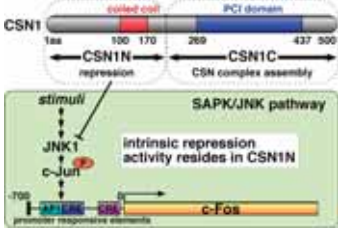
Survival of a life-form largely depends on how it perceives the environmental information and what it does in response. Higher plants, unlike many animals, spend most of their lives immobilized at a single location. Consequently, plants have acquired a high degree of plasticity during evolution, giving unique regulation of morphogenesis in response to environmental signals. Among such signals, evidently, light is the key signal for plant development and survival. A genetic screen with *Arabidopsis thaliana* has revealed a set of *cop/det/fus* mutants possessing light grown seedling phenotype in the dark. The ten responsible *COP/DET/FUS* genes are negative regulators of photomorphogenesis in darkness. Among these genes, six code for the subunit proteins of a large nuclear protein complex called the COP9 Signalosome (CSN).

CSN is not specific to plants but highly conserved in a broad range of animals where light signal has less influence on morphogenesis. Interestingly, each of the eight subunits of the CSN has structural conservation with one of the eight subunits in the Lid-subcomplex of the 26S Proteasome, the machinery for ubiquitin-mediated protein degradation. These two complexes are paralogous to each other, suggesting an evolutional relationship (left figure). Individual subunits of the CSN has been linked to various signal pathways leading to gene expression regulation and cell cycle control.



We previously found that CSN interacts with the E3 ubiquitin ligases and regulates the ubiquitin-proteasome degradation pathway by releasing the covalent bond between a small ubiquitin-like protein (plant Rub1 or animal NEDD8) and the Cullin subunit of the E3 ligase [1]. This regulation through protein degradation eventually modulates the signal transduction pathways involved in cell cycle, morphogenesis, and stress response [2]. Further dissection revealed that the C-terminal half of CSN1 subunit, encompassing the PCI domain, is responsible for interaction with CSN2, CSN3, and CSN4 subunits and therefore required as a scaffold for CSN assembly as a complex. The N-terminal portion of CSN1 (CSN1N), on the other hand, possessed an intrinsic function of repression on gene expression. We found that CSN1N represses the SAPK/JNK (c-Jun N-terminal kinase) signaling pathway by suppressing the activated signal of the proto-oncogene c-Fos through its responsive elements on the promoter [3].

We further found that CSN1N potently reduces the accumulation of JNK1 protein in the cells leading to the suppression of c-Jun phosphorylation (right figure). However, this regulation of repression was independent from the known protein-degradation mediated regulation, as shown by several lines of circumstantial evidence. In belief that this regulation was caused through protein-protein interaction, we set out to identify interacting proteins that directly bind to CSN1N. We have isolated and identified a novel spectrum of interacting proteins (NBPs) supporting the above idea. To understand the biological roles of NBPs in the regulation of signal transduction on morphogenesis, we have constructed a series of knockout plants defective in each of the NBP gene, taking advantage of the various resources available in *Arabidopsis*. Detailed biochemical analyses on the CSN-NBP interactions in combination with *in planta* research will reveal the novel mechanism of CSN on signal transduction.



1. *Science* **292**, 1382-5 (2001). 2. *Curr Biol* **12**, 667-72 (2002). 3. *J Mol Biol* **305**, 1-9 (2001).

Role of phospholipase D in plant cell morphogenesis, Yamamoto Y, Ohashi Y, Oka A, Aoyama T; *Arabidopsis* AtCycA2; 3 is a key regulator of termination system of endoreduplication, Imai K *et al*., 2004 Ann Meeting of Mol Biol Soc Jpn, 8 - 11 December (Kobe).

Grants

Oka A, Molecular basis of cytokinin signaling in plant cells, Grant-in-Aid for Scientific Research on Priority Areas (A), 1 April 2003 - 31 March 2005; Two-component regulatory system of phosphorelay involved in cytokinin signaling, Grant-in-Aid for Scientific Research (B), 1 April 2004 - 31 March 2007.

Aoyama T, Molecular mechanism of adaptive responses controlled by *Arabidopsis* His-Asp phosphorelay signal transduction, Grant-in-Aid for Scientific Research on Priority Areas (B), 1 April 2000 - 31 March 2005; Roles of phospholipid signaling in root-hair formation, Grant-in-Aid for Scientific Research (B), 1 April 2004 - 31 March 2007.